



Anti-diabetic Activity of *Chrysophyllum albidum* (G. Don) Stem Bark in Alloxan-induced Type 1 Diabetic Female Wistar Rats

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Abstract

Anti-diabetic activity of aqueous extract of *Chrysophyllum albidum* stem bark (AECASB) in female Wistar rats was investigated to confirm or refute the purported use and the underlying mechanism of action of *C. albidum* stem bark in the management of type 1 diabetes in Nigeria traditional medicine. Seventy female rats (180.60 ± 8.50 g) were assigned into seven groups. Rats in Group 1 (non-diabetic) were orally administered 1 mL of distilled water, while animals in Groups 2-7 were made diabetic and orally administered 1 mL of distilled water, 2.5 mg/kg body weight (bwt) of glibenclamide, 25, 50, 100 and 200 mg/kg bwt of AECASB, once daily for 14 days, respectively. Fasting blood glucose (FBG) levels of the rats were determined on days 0, 1, 4, 7, 10 and 14. Other biochemical, hematological parameters and pancreas histology of the rats were also determined/examined. The 25 mg/kg bwt of AECASB produced the most significant ($p < 0.05$) reversal on the alloxan treatment-induced increases in FBG, biochemical and hematological parameters and regenerated the pancreas. In conclusion, the 25 mg/kg bwt of aqueous extract of *C. albidum* stem was the effective in the management of diabetes and might have acted via regeneration of the pancreas, enhancement of glucose utilization and reduction of blood glucose.

Keywords: Type 1 diabetes mellitus, fasting blood glucose, *Chrysophyllum albidum*, Sapotaceae

Introduction

Diabetes mellitus is a protracted metabolic ailment caused by a relative lack of insulin and/or reduced insulin activity (Ponugoti et al. 2013). In recent times, there has been increased prevalence of diabetes in all the regions of the world as 463 million people are currently living with diabetes and the incidence is projected to rise to 700 million by 2045 (IDF 2019). In Africa region alone, 143% increase in diabetes mellitus is projected for 2045. Type 1 diabetes though may be developed at any stage in life, occurs most frequently in children and adolescents and accounts for 1.1 million global

diabetics below 20 years of age (Sidorchuk 2003). An estimated 25,800 children and adolescents are believed to be living with type 1 diabetes in Africa. However, in countries such as Nigeria where there is limited access to insulin and inadequate health service provision, diabetic children and adolescents suffer terrible complications and early death.

The available oral anti-diabetic agents are of low potency, relatively expensive with several side effects (Ganesan and Sultan 2019). This necessitated the search for more effective, relatively cheap and safer medications for the management of diabetes. To this effect, a

number of plants have been used locally for the management of diabetes without scientific evidence on the efficacy and safety of the plant parts used in the open scientific literature. One of such plants is *Chrysophyllum albidum*.

Chrysophyllum albidum G. Don., also called African star apple (English), belongs to the Sapotaceae family (Adebayo et al. 2010). It is also known by different names in Nigeria as *agbalumo* (Yoruba), *udara* (Igbo), *agbaluba* (Hausa) and *eha* (Ebira) (Orijajogun et al. 2013). It is a tropical fruit tree that is widely distributed in the low land rain forest zones of Africa and grows up to 25–37 m high (Madubuike and Ogbonnaya 2003).

The plant parts are used locally for the management of various disease conditions. The leaf is used as an emollient and for handling indigestion and diarrhoea (Adisa 2000). The leaf and seed cotyledons are used as lotions in the treatments of vaginal and dermatological taints in Western Nigeria (Adewusi and Bada 1997). The roots, barks and leaves of *C. albidum* are applied topically to bruises and cuts in southern Nigeria (Okoli and Okere 2010). The seed and root extracts are used to arrest bleeding from fresh injuries and to inhibit microbial growth (Okoli and Okere 2010), while the stem bark is used for the treatments of yellow fever and malaria (Bello and Henry 2015). Various parts of the plant have also been touted to be used in managing diabetes in folk medicine of Nigeria (Houessou et al. 2012).

Ibrahim et al. (2019) reported that *C. albidum* fruit-skin supplemented diet lowered blood glucose, glycosylated haemoglobin, lipid profile and increased body weight, insulin, hepatic glycogen and red blood cell levels in streptozotocin-induced diabetic rats. Ehigiator and Adikwu (2019) after investigating the effects of the ethanolic extract of *C. albidum* stem bark on alloxan-induced diabetic rats concluded that the extract reduced fasting blood glucose, total cholesterol, triglycerides, superoxide dismutase, catalase, glutathione and increased high density lipoprotein and malondialdehyde. Olanudun et al. (2018)

reported that lupeol-3-acetate contained in the methanolic extract of *C. albidum* stem bark was responsible for the anti-hyperglycaemic effect of the extract. Furthermore, Adebayo et al. (2010) concluded that the ethanolic leaf extract of *C. albidum* could be employed as a booster of natural antioxidants for the treatment of free radical induced-oxidative stress disorders. The ethanolic root bark extract of *C. albidum* have also been reported to exhibit anti-hyperglycaemic, hepatoprotective and free radical scavenging activities in alloxan-induced diabetic rats (Onyeka et al. 2012).

Despite the widely reported antidiabetic activity of various parts of *C. albidum* in the open scientific literature, there is still insufficient comprehensive report on the anti-diabetic activity of the *C. albidum* stem bark in alloxan-induced type 1 diabetic female Wistar rats and its possible mode of action as an antidiabetic agent. Therefore, this study was aimed at providing a more comprehensive investigation on the antidiabetic activity of aqueous extract of *Chrysophyllum albidum* stem bark and its likely mode of action in alloxan-induced type 1 diabetic female Wistar rats.

Materials and Methods

Plant material and authentication

Chrysophyllum albidum, obtained in March, 2016, from Ganmo in Ifelodun Local Government Area of Kwara State was authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Nigeria. A voucher specimen (UILH/001/1170) was deposited at the Herbarium Unit of the Department. The plant name, *Chrysophyllum albidum* (G. Don). was checked (data supplied on 2012-03-26) on <http://theplantlist.org/tpl1.1/record/kew-39944> and was found to be an accepted name.

Experimental animals

Seventy Wistar rats (180.60 ± 8.50 g) were obtained from the Animal House of the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria.

Drugs, assay kits, chemicals and automated hematological analyzer

Glibenclamide was a product of Medico Remedies Pvt Ltd., Juhu, Mumbai, India, while the assay kits for urea, creatinine, albumin and glucose-6-phosphate dehydrogenase were products of Randox Laboratories Ltd., Co-Antrim, United Kingdom. Alloxan monohydrate was a product of Sigma Chemical Company, St. Louis, Mo, USA. The automated hematological analyzer, SYSMEX KX21, was manufactured by SYSMEX Corporation, Harrier, Japan.

Preparation of aqueous extract of *C. albidum* stem bark

The method described by Yakubu et al. (2005) was adopted for the preparation of AECASB with some modifications. The stem bark was cut into pieces, and oven-dried at 40 °C to a constant weight. The powdered stem bark (2000 g) was extracted in 5 L of distilled water for 72 hours at 25 °C. The resulting filtrate which was freeze-dried (Labconco Freeze Drier, Model 64132, Kansas City, Missouri, USA) gave a percentage yield of 3.76%. The AECASB was reconstituted in distilled water to give the desired doses of 25, 50, 100, 200

mg/kg body weight (to simulate local usage) used for the study.

Induction of type 1 diabetes mellitus

The fasting blood glucose levels of overnight fasted animals were determined before the induction of type 1 diabetes as described by Sharma et al. (2010) with slight modifications. The rats were induced into diabetes with a single intraperitoneal injection of 120 mg/kg body weight of alloxan monohydrate. Blood glucose levels of the rats were also determined 72 hours after alloxan administration. Blood samples were drawn from the sharply cut tail vein of the rats and placed on the test strip that had been inserted into the glucometer (Roche Diagnostic, Mannheim, Germany). Animals with blood glucose levels equal to or greater than 250 mg/dL were considered diabetic and used for the subsequent experiments (Oloyede et al. 2015).

Animal grouping

A total of seventy female Wistar rats were used in this study. The animals were randomly assigned into seven groups (1-7) of ten animals each as shown in Table 1.

Table 1: Animal groups and treatments

Groups	Treatments
1	Non-diabetic rats +1 mL of distilled water
2	Diabetic rats + 1 mL of distilled water
3	Diabetic rats + 2.5 mg/kg bwt of glibenclamide
4	Diabetic rats + 25 mg/kg bwt of aqueous extract of <i>C. albidum</i> stem bark
5	Diabetic rats + 50 mg/kg bwt of aqueous extract of <i>C. albidum</i> stem bark
6	Diabetic rats + 100 mg/kg bwt of aqueous extract of <i>C. albidum</i> stem bark
7	Diabetic rats + 200 mg/kg bwt of aqueous extract of <i>C. albidum</i> stem bark

Ethical clearance

The study was carried out after ethical approval from the University of Ilorin Ethical Review Committee approval number UERC/ASN/2017/907 of 12th June 2017.

Preparation of serum, tissue supernatant and determination of biochemical and hematological parameters

The procedure described by Yakubu et al. (2008) was adopted for the preparation of serum and tissue supernatant.

The procedures described by Bernfield (1955), Passoneau and Lauderdale (1974), Brandstrup

et al. (1957), Noltmann et al. (1961), Oboh et al. (2010), Fawcett and Scott (1960), Bartels et al. (1972), Dumas et al. (1971), Burtis and Ashwood (1999) and Fiske and Subbarow (1921) were adopted for the determination of hepatic glucose, hepatic glycogen, hepatic glucokinase, glucose-6 phosphate dehydrogenase, α -amylase, serum urea, creatinine, albumin, electrolytes (sodium, potassium, bicarbonate and chloride ions) and phosphate ions, respectively. Hematological parameters of red blood cells (RBC), haemoglobin (Hb) and packed cell volume (PCV) were determined using the Sysmex automated haematology analyzer.

Histological examination of the tissues

The method highlighted by Drury and Wallington (1980) was adopted for the preparation of the pancreatic photomicrographs. Briefly, pancreas specimens were separately fixed in 10% formalin and dehydrated in ascending grades of ethanol (70%, 90% and 95%), cleaned in xylene, and embedded in paraffin wax (melting point 56 °C) (Krauss 2001). The tissues were then sectioned (5 μ m thick) and stained with Hematoxylin and Eosin stain (H & E). The histological slides were examined using Acuscope® (China) microscope with a TSVIEW® Software (China) to observe the pathological changes. Cross section of the liver and kidney were captured at $\times 400$, while that of the pancreas was captured at $\times 100$ with Canon Image Folio package software (Model: Powershot A2500, Japan).

Data analysis

Data were expressed as the mean \pm SEM of ten replicates. Data were analyzed using one-way analysis of variance followed by Tukey's *post-hoc* test for multiple comparisons. Statistical analyses were done with Graph Pad Statistical Package version 6.0 at 95% confidence level.

Results

Table 2 shows fasting blood glucose (FBG) levels of diabetic rats orally administered AECASB for 14 days. The FBG levels of the rats administered alloxan significantly ($p < 0.05$) increased from 80.00 ± 2.94 mg/dl to 506.00 ± 10.30 mg/dl. By the 10th day of the experiment, the FBG levels of alloxan-induced diabetic rats administered 25 mg/kg bwt of AECASB were not significantly ($p > 0.05$) different from the distilled water treated non-diabetic rats, and this was sustained till the end of the experimental period. Interestingly, the FBG levels of alloxan treated rats that received the 50, 100 and 200 mg/kg bwt of AECASB compared favorably with the distilled water treated non-diabetic rats on day 14 just like that of the alloxan treated rats that received glibenclamide (Table 2).

Administration of alloxan monohydrate significantly ($p < 0.05$) increased the hepatic glucose concentrations and decreased the hepatic glycogen levels when compared with distilled water treated non-diabetic rats (Figure 1). However, administration of AECASB at 25 mg/kg bwt of AECASB reversed the alloxan treatment-induced increase in the hepatic glucose and decrease in the hepatic glycogen, while the 50, 100 and 200 mg/kg bwt treatments were not significantly ($p > 0.05$) different from the distilled water treated diabetic rats.

Compared with the distilled water treated non-diabetic rats, administration of alloxan significantly ($p < 0.05$) decreased the activity of glucokinase by 42.8% (Figure 2) whereas administration of all the doses of AECASB significantly ($p < 0.05$) increased the glucokinase activity. However, the 25 mg/kg bwt treatment compared well with the distilled water treated non-diabetic rats, whereas the treatments with the 50, 100 and 200 mg/kg bwt of AECASB were not different from the diabetic rats that were administered glibenclamide.

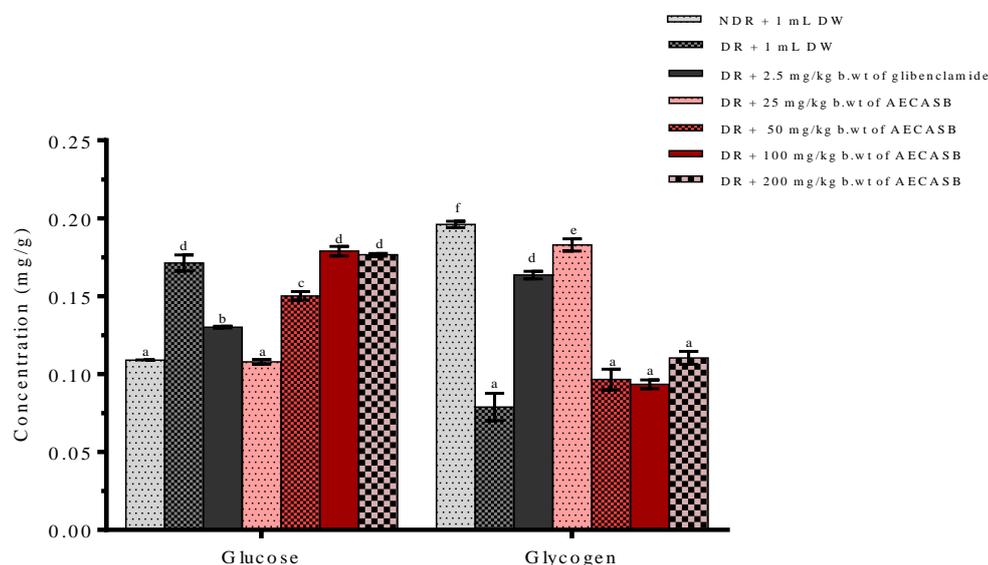


Figure 1: Hepatic glucose and glycogen levels of diabetic female rats orally administered aqueous extract of *Chrysophyllum albidum* stem bark. Where: NDR = Non-diabetic rats; DR= Diabetic rats; AECASB = Aqueous extract of *C. albidum* stem bark; DW = Distilled water.

Although administration of AECASB at all the doses reversed the alloxan-induced significant ($p < 0.05$) decrease in glucose-6-phosphate dehydrogenase activity (Figure 3), the activity of the enzyme in the diabetic animals administered 25 and 50 mg/kg bwt of AECASB was not significantly ($p > 0.05$) different from that of the distilled water treated non-diabetic rats, whereas the 100 and 200 mg/kg bwt treatments were not significantly ($p > 0.05$) different from the glibenclamide treated diabetic rats.

Compared with the distilled water treated non-diabetic rats, alloxan administration significantly ($p < 0.05$) increased α -amylase activity in the small intestine, pancreas and liver of the female rats (Figure 4). Although, the activity of α -amylase in the tissues significantly ($p < 0.05$) reduced after the oral administration of AECASB, the 25 mg/kg bwt of the AECASB produced α -amylase activity that was not significantly different from the distilled water treated non-diabetic rats in all the tissues. Furthermore, the hepatic α -amylase

activity produced by all the doses of AECASB compared well ($p > 0.05$) with that of non-diabetic control rats that received distilled water.

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Table 2: Fasting blood glucose levels of diabetic female rats orally administered aqueous extract of *Chrysophyllum albidum* stem bark

Group/ Day	NDR + 1 mL of distilled water	DR + 1 mL of distilled water	DR+ 2.5 mg/kg bwt of Glibenclamide	DR+ 25 mg/kg bwt of AECASB	DR + 50 mg/kg bwt of AECASB	DR+ 100 mg/kg bwt of AECASB	DR+ 200 mg/kg bwt of AECASB
0	80.50 ± 4.04 ^a	80.00 ± 2.94 ^a	74.75 ± 6.80 ^a	78.25 ± 8.42 ^a	75.75 ± 4.27 ^a	69.75 ± 9.85 ^a	73.75 ± 6.45 ^a
1	76.50 ± 8.43 ^a	344.25 ± 59.66 ^b	394.00 ± 104.76 ^b	356.75 ± 59.87 ^b	368.50 ± 88.75 ^b	389.00 ± 12.27 ^b	364.00 ± 86.47 ^b
4	79.00 ± 5.60 ^a	384.75 ± 56.99 ^c	335.25 ± 100.81 ^c	239.50 ± 44.80 ^b	335.50 ± 91.78 ^c	300.00 ± 24.39 ^c	299.50 ± 28.97 ^c
7	80.00 ± 5.94 ^a	390.00 ± 16.99 ^b	274.25 ± 79.40 ^d	109.75 ± 9.54 ^b	261.75 ± 54.72 ^c	233.25 ± 42.37 ^c	252.50 ± 71.82 ^c
10	76.00 ± 6.33 ^a	432.00 ± 48.08 ^c	126.00 ± 26.14 ^b	70.00 ± 4.69 ^a	117.00 ± 11.60 ^b	102.50 ± 10.25 ^b	110.75 ± 8.26 ^b
14	73.00 ± 6.33 ^a	506.00 ± 10.30 ^d	82.25 ± 6.90 ^a	70.00 ± 3.74 ^a	80.00 ± 3.37 ^a	85.50 ± 5.57 ^a	84.00 ± 12.99 ^a

Values are means of ten replicates ± SEM; values with different superscripts on the same row are significantly different.

Where: NDR = Non-diabetic rats; DR = Diabetic rats; AECASB = Aqueous extract of *C. albidum* stem bark; DW = Distilled water.

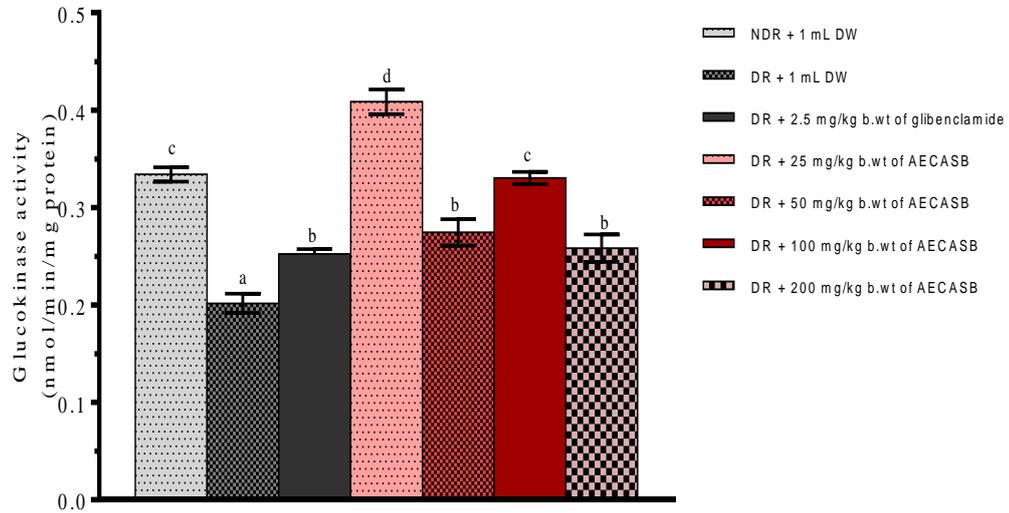


Figure 2: Hepatic glucokinase activity of diabetic female rats orally administered aqueous extract of *Chrysophyllum albidum* stem bark. Where: NDR = Non-diabetic rats; DR= Diabetic rats; AECASB = Aqueous extract of *C. albidum* stem bark; DW = Distilled water.

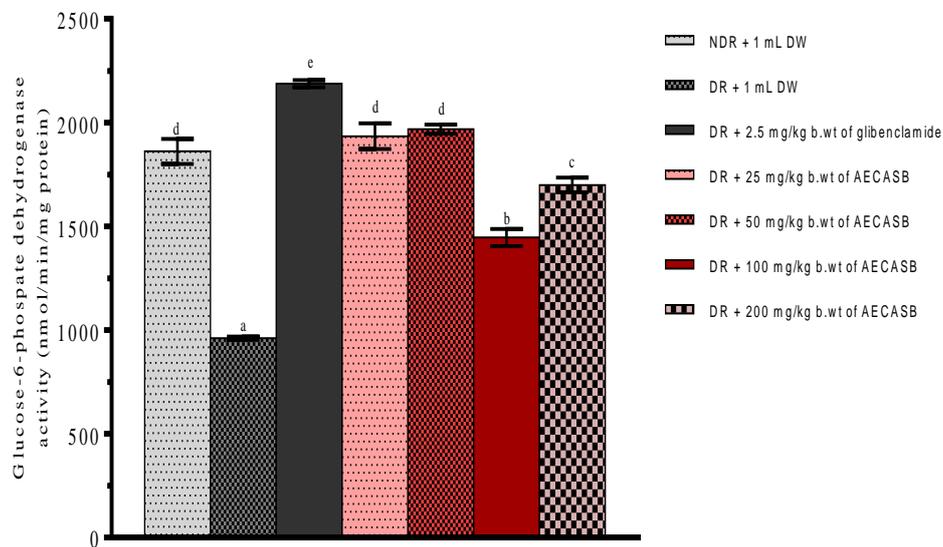


Figure 3: Hepatic glucose-6-phosphate dehydrogenase activity of diabetic female rats orally administered aqueous extract of *Chrysophyllum albidum* stem bark. Where: NDR = Non-diabetic rats; DR= Diabetic rats; AECASB = Aqueous extract of *C. albidum* stem bark; DW = Distilled water.

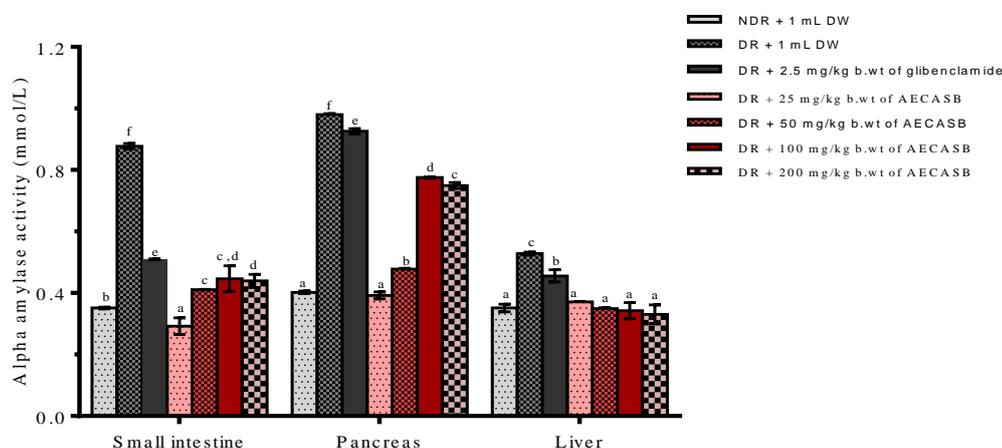


Figure 4: Alpha amylase activity of diabetic female rats orally administered aqueous extract of *Chrysophyllum albidum* stem bark. Where: NDR = Non-diabetic rats; DR= Diabetic rats; AECASB = Aqueous extract of *C. albidum* stem bark; DW = Distilled water.

Administration of alloxan significantly ($p < 0.05$) increased the serum urea and creatinine concentrations (Figure 5) and decreased the albumin levels (Figure 6) when compared with the distilled water treated non-diabetic rats. Administration of all the doses of AECASB

significantly ($p < 0.05$) decreased the levels of urea and creatinine, but the 25 mg/kg bwt of AECASB produced urea and creatinine levels that compared favorably ($p > 0.05$) with the non-diabetic control rats.

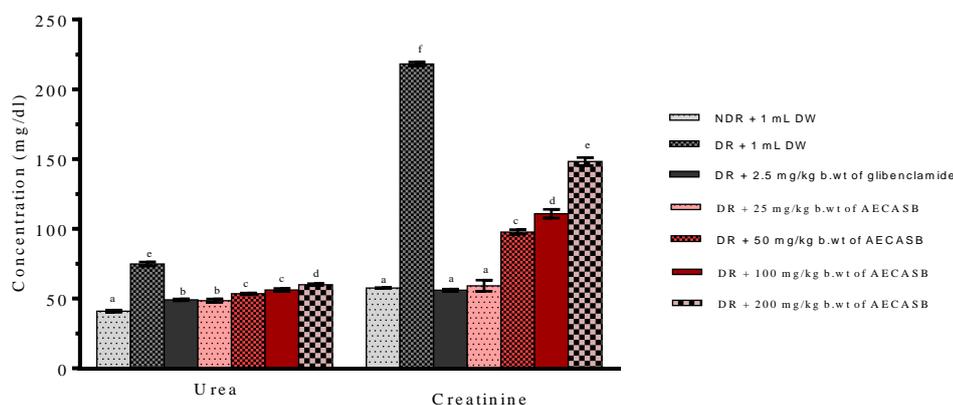


Figure 5: Serum urea and creatinine concentrations of diabetic female rats orally administered aqueous extract of *Chrysophyllum albidum* stem bark. Where: NDR = Non diabetic rats; DR= Diabetic rats; AECASB = Aqueous extract of *C. albidum* stem bark; DW = Distilled water.

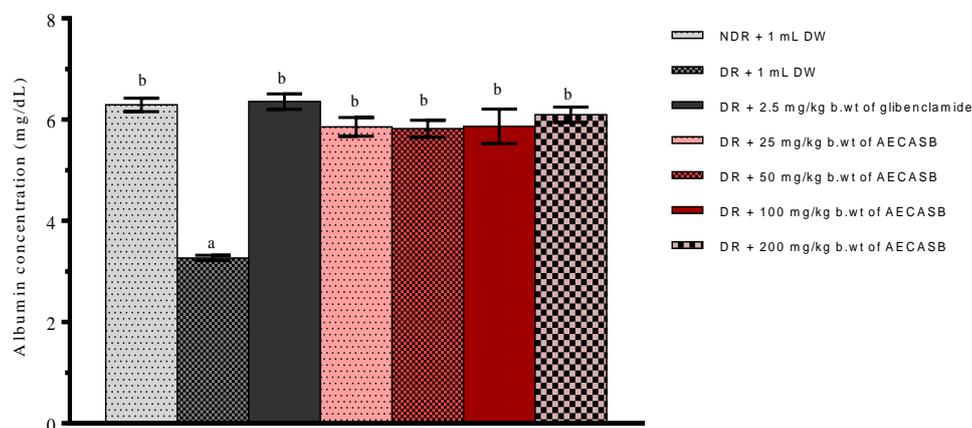


Figure 6: Serum albumin concentrations of diabetic female rats orally administered aqueous extract of *Chrysophyllum albidum* stem bark. Where: NDR = Non diabetic rats; DR= Diabetic rats; AECASB = Aqueous extract of *C. albidum* stem bark; DW = Distilled water.

Alloxan administration significantly ($p < 0.05$) reduced the levels of serum potassium, chloride, PCV, Hb, RBC and increased the levels of carbonate and phosphate ions when compared with the distilled water treated non-diabetic rats (Tables 3 and 4). Administration of all the doses of AECASB significantly ($p < 0.05$) increased the alloxan-induced related decrease in haematological and electrolyte parameters with only the 25 mg/kg bwt of AECASB producing values that compared favorably ($p > 0.05$) with the non-diabetic distilled water treated rats.

Histological examinations of the pancreas of distilled water treated non-diabetic rats revealed no visible lesion (Plate 1) whereas the parenchyma and the islet of the pancreas of distilled water treated diabetic rats was completely obliterated (Plate 2). Treatment of diabetic rats with 2.5 mg/kg bwt of glibenclamide revealed necrotic pancreas with loose connective and adipose tissues (Plate 3) whereas that of 25 mg/kg bwt of AECASB was devoid of any visible lesion (Plate 4). Furthermore, the pancreas of the rats administered 50, 100 and 200 mg/kg bwt of AECASB (Plates 5, 6 and 7) revealed several pathological conditions like severe congestion of the vessels, parenchyma that were almost

taken over by adipose tissue, small sized lobular portion of the pancreatic acini, and in addition to the loose, irregular connective tissues that were severely infiltrated in the diabetic rats that separately received 50 and 100 mg/kg bwt of AECASB (Plates 5 and 6). The pancreas of the diabetic rats treated with 200 mg/kg bwt of AECASB showed severe necrosis of the pancreatic cells, loose adipose and connective tissues (Plate 7).

Discussion

Alloxan is one of the chemicals commonly employed to induce type 1 diabetes mellitus (Onyeka et al. 2018). It has been reported to cause massive selective destruction and reduction of beta cells of the islets of Langerhans mediated by the formation of reactive oxygen species (Szkudelski 2001), resulting in partial or complete loss of insulin synthesis resulting in hyperglycemia (Nagappa et al. 2003). The reference antidiabetic drug, glibenclamide, is a sulfonylurea that inhibits the ATP-sensitive potassium channels in the pancreatic beta cells and enhance glucose utilization in the target tissues (Leonard et al. 2017).

Table 3: Hematological parameters of diabetic female rats orally administered aqueous extract of *Chrysophyllum albidum* stem bark

Parameters/Group	NDR + 1 mL of distilled water	DR + 1 mL of distilled water	DR + 2.5 mg/kg bwt of glibenclamide	DR+ 25 mg/kg bwt of AECASB	DR + 50 mg/kg bwt of AECASB	DR + 100 mg/kg bwt of AECASB	DR + 200 mg/kg bwt of AECASB
RBC ($\times 10^6$)	6.27 \pm 0.15 ^c	3.72 \pm 0.13 ^a	5.53 \pm 0.28 ^b	5.10 \pm 0.51 ^b	5.50 \pm 0.14 ^b	6.40 \pm 0.22 ^c	6.25 \pm 0.38 ^c
Hb (g/dl)	11.00 \pm 0.10 ^c	5.95 \pm 0.25 ^a	9.30 \pm 0.50 ^b	8.90 \pm 0.10 ^b	9.50 \pm 0.20 ^b	10.05 \pm 0.55 ^b	9.55 \pm 0.35 ^b
PCV (%)	37.30 \pm 0.20 ^c	17.40 \pm 0.70 ^a	32.15 \pm 0.35 ^b	31.25 \pm 0.55 ^b	33.05 \pm 1.15 ^b	30.95 \pm 0.55 ^b	31.70 \pm 0.70 ^b

Values are means of ten replicates \pm SEM and values with different superscripts on the same row are significantly different.

Where: NDR = Non-diabetic rats; DR= Diabetic rats; AECASB = Aqueous extract of *C. albidum* stem bark

Table 4: Serum electrolytes of diabetic female rats orally administered aqueous extract of *Chrysophyllum albidum* stem bark

Parameters/Group	NDR + 1 mL of distilled water	DR + 1 mL of distilled water	DR + 2.5 mg/kg bwt of glibenclamide	DR+ 25 mg/kg bwt of AECASB	DR + 50 mg/kg bwt of AECASB	DR + 100 mg/kg bwt of AECASB	DR + 200 mg/kg bwt of AECASB
Sodium	14.13 \pm 0.91 ^c	6.38 \pm 0.64 ^a	9.21 \pm 0.71 ^b	13.84 \pm 1.11 ^c	10.01 \pm 1.06 ^b	9.34 \pm 0.87 ^b	9.44 \pm 0.64 ^b
Potassium	6.37 \pm 0.24 ^c	2.18 \pm 0.22 ^a	4.21 \pm 0.16 ^c	5.44 \pm 0.11 ^d	3.94 \pm 0.26 ^b	3.71 \pm 0.27 ^b	4.01 \pm 0.06 ^c
Chloride	9.48 \pm 1.06 ^c	4.11 \pm 0.38 ^a	6.24 \pm 0.26 ^b	8.85 \pm 0.91 ^c	5.92 \pm 0.13 ^b	6.10 \pm 0.12 ^b	5.89 \pm 0.20 ^b
Carbonate	3.81 \pm 0.04 ^a	5.96 \pm 0.21 ^b	3.51 \pm 0.22 ^a	3.91 \pm 0.03 ^a	6.01 \pm 0.11 ^b	5.81 \pm 0.04 ^b	5.73 \pm 1.26 ^b
Phosphate	8.17 \pm 0.24 ^c	3.11 \pm 0.24 ^a	5.01 \pm 0.33 ^b	8.02 \pm 0.24 ^c	7.92 \pm 0.22 ^c	7.81 \pm 1.02 ^c	3.21 \pm 0.14 ^a

Values are means of ten replicates \pm SEM and values with different superscripts on the same row are significantly different.

Where: NDR = Non-diabetic rats; DR= Diabetic rats; AECASB = Aqueous extract of *C. albidum* stem bark.

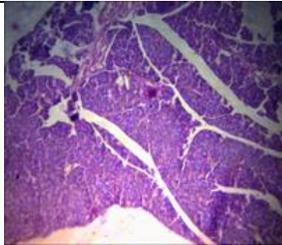


Plate 1: Cross section of pancreas of non-diabetic rat administered 1 ml of distilled water (x 100; H & E)

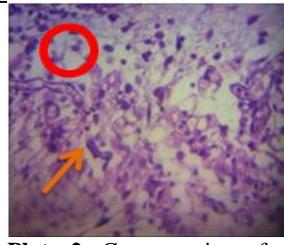


Plate 2: Cross section of pancreas of diabetic rat administered 1 ml of distilled water (x 100; H; & E). ○: Loose adipose and connective tissues; →: Inflammatory cell

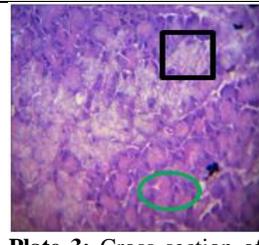


Plate 3: Cross section of pancreas of diabetic rat administered 2.5 mg/kg body weight of glibenclamide (x 100; H & E). : Loose adipose and connective tissues; ○: Necrosis

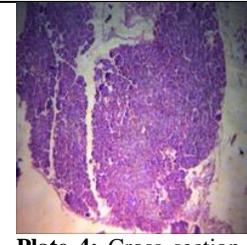


Plate 4: Cross section of pancreas of diabetic rat administered 25 mg/kg body weight of the extract (x 100; H & E).



Plate 5: Cross section of pancreas of diabetic rat administered 50 mg/kg body weight of the extract (x 100; H & E). : infiltrated loose irregular connective tissues.



Plate 6: Cross section of pancreas of diabetic rat administered 100 mg/kg body weight of aqueous extract of *C. albidum* stem bark (x 100 H & E)

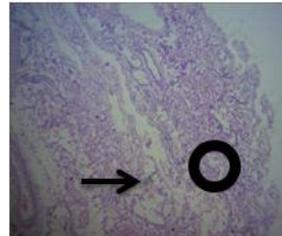


Plate 7: Cross section of pancreas of diabetic rat administered 200 mg/kg body weight of aqueous extract of *C. albidum* stem bark (x 100 H & E). →: Loose adipose tissue; : Severe

Fasting blood glucose is an important indicator of diabetic status. The increase in fasting blood glucose levels of distilled water treated diabetic rats in this study may be attributed to reduction or lack of insulin production by the beta cells of the pancreas which might have been resulted from the destruction of the beta cells of the pancreas. This agrees with the findings of Mnonopi et al. (2012), Ozougwu et al. (2013) and Yakubu and Ogunro (2014) who attributed the increase in fasting blood glucose levels in diabetic animals to beta cell destruction. The reduction of the alloxan-related elevation in the fasting blood glucose levels of the diabetic rats following AECASB administration suggests that it has anti-diabetic property and might have acted by optimizing glucose utilization and stimulating pancreas regeneration. The findings here agrees with that of previous studies that attributed decrease in fasting blood glucose levels of diabetics treated with various oral anti-diabetic agents to pancreas regeneration (Asagba et al. 2019, Ibrahim et al. 2019) and improved glucose utilization by the muscle cells and the hepatocyte (Adebayo et al. 2010, Petersen et al. 2017). Furthermore, the present study recorded the highest anti-hyperglycaemic activity at lowest dose investigated as against those reported by Adebayo et al. (2010) and Ehigiator and Adikwu (2019) who reported most potent anti-hyperglycaemic activity at higher doses.

Glucose is the primary source of energy that is stored mainly in the liver and skeletal muscles (Ho 2018). Cellular glucose concentration is tightly regulated to prevent persistent hyperglycemia and conserve sugar in the form of glycogen for further uses (Gusarov and Nudler 2018). However, the inability of the body to regulate glucose due to pancreas destruction/insensitivity of the skeletal muscles or hepatocytes to insulin results in diabetes mellitus. The increase in hepatic glucose accompanied with decreased glycogen content in the distilled water treated diabetic rats as recorded in the present study, might be due to pancreas destruction. The reduction in alloxan-

induced exacerbated hepatic glucose levels and increase in glycogen content following AECASB administrations signifies restoration of the homeostasis function of the liver.

Glucokinase, a rate limiting enzyme commonly stimulated by insulin, facilitates glucose utilization by converting glucose to glucose-6-phosphate in the liver. The decrease in the activity of this enzyme in the distilled water treated diabetic rats might be due to lack of insulin owing to pancreas destruction as evident in this study. The increase in glucokinase activity of AECASB treated diabetic rats might be due to activation of glycolysis stimulated by pancreas regeneration. This suggests that AECASB demonstrated its anti-hyperglycaemic effects by enhancing glucose utilization through activation of glycolysis and pentose phosphate pathways. This finding has been substantiated by other investigators including (Eze et al. 2016) who attributed the increase in glucokinase activity to enhanced glucose utilization.

Hyperglycemia has been reported to trigger a decrease in the activity of a glucose-6-phosphate dehydrogenase in the pentose phosphate pathway (Srinivasan et al. 2014). This might be responsible for the recorded decrease in the glucose-6-phosphate dehydrogenase activity of the distilled water treated diabetic rats in this study. The increase in the activity of the enzyme following AECASB administration is an indication that it might suppress oxidative stress associated with diabetes and enhance glucose utilization via pentose phosphate pathway. The significant decrease in α -amylase activity signifies delay in glucose absorption and moderates postprandial blood sugar level which plays a central role in the development and progression of diabetic complications (Sabiu and Ashafa 2016). The mechanism of action of anti-diabetic activity of *Chrysophyllum albidum* stem bark might be through increased utilization of glucose by peripheral tissues, increased synthesis of hepatic glycogen, inhibition of alpha amylase, regeneration of the

pancreas and inhibition of hepatic glucose production (Krishnasamy et al. 2016).

The kidney is an important organ in glucose homeostasis (Abe and Kalantar-Zadeh 2015) and may be affected by beta-cell dysfunction (Koppe et al. 2016). Elimination of urea and creatinine from the plasma is one of the functions of kidney and is normally used for the assessment of renal competence (Koppe et al. 2016). The increase in urea concentrations of the distilled water treated diabetic rats might be due to deamination of glucogenic amino acids and increase muscle proteolysis induced by alloxan treatment. The finding in the present study with respect to urea levels is in line with those previously reported by Adebayo et al. (2010) and Sunmonu and Afolayan (2013) who attributed elevated serum urea and/or creatinine of diabetic subjects to beta cell dysfunction.

The concentration of creatinine normally is constant but becomes elevated when renal function is impaired. The increase in creatinine levels of the distilled water treated diabetic rats might be due to impairment of renal functions ensuing from hyperglycaemia. A disturbance in electrolytes is associated with diabetes mellitus, as some electrolytes play essential roles in intermediary metabolism and cellular functions including osmosis and acid-base balance (Ogunleye and Asaolu 2016). Their deficiency is associated with shift of osmotic fluids due to hyperglycemia or total body deficits due to osmotic diuresis. Glucose is an osmotically active substance, so its osmolarity increases in hyperglycemic states, leading to movement of water out of the cells which signals symptoms such as polyuria, polydipsia and polyphagia that may lead to impediments (IDF 2019). The decrease in the levels of urea and creatinine, reversal of hypotonic renal losses and hypophosphatemia in the AECASB-treated diabetic rats might be due to improvement in insulin release potentiated by pancreas regeneration. The restoration of electrolytes to near normal by the 25 mg/kg bwt of AECASB suggests that it might prevent or ameliorate hyperglycaemia-induced renal dysfunction associated with diabetes mellitus

and improve glucose utilization by regenerating the pancreas for insulin secretion which in turn promotes the transportation of glucose and its phosphorylation in the muscle and liver cells (Liamis et al. 2014). This agrees with the previous reports of Ibrahim et al. (2019), and Yakubu and Ogunro (2014) who attributed reduction of the alloxan-induced increased in biochemical parameters to ameliorative potential of the plant extracts.

Diabetes is associated with an upset of haematological parameters that may result in anaemia as one of the complications in diabetic chemotherapy (Eze et al. 2016, Eze et al. 2019). The decrease in red blood cell parameters (red blood cell count, haemoglobin and pack cell volume) in the distilled water treated diabetic rats might be due to glycosylation of glucose with the red blood cell and haemoglobin triggered by hyperglycaemia. The osmotic effect of glucose which results not only in dwindled circulating blood volume and fluid shift from the intracellular spaces causing cellular dryness, but also in glycosylation of haemoglobin (Khanduker et al. 2017, Yakubu and Ogunro 2014). The reversal of the decreased erythrocytic indices might be due to recovery from alloxan assault and capable of ameliorating erythrocytic disorder associated with diabetes as evident in this study. This contradicts the findings of Adebayo et al. (2010) who reported non-significant difference in red blood cell parameters of diabetic Wistar rats.

Conclusion

Aqueous extract of *C. albidum* stem bark at 25 mg/kg body weight was effective in the management of diabetes mellitus and might have acted via regeneration of the pancreas, enhancement of glucose utilization and reduction of blood glucose. It also ameliorated renal dysfunction associated with diabetes mellitus. Further study might be needed to identify the active principles responsible for the anti-diabetic activity.

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